# Release of Cyanobacterial Metabolites Due to Preoxidation Processes

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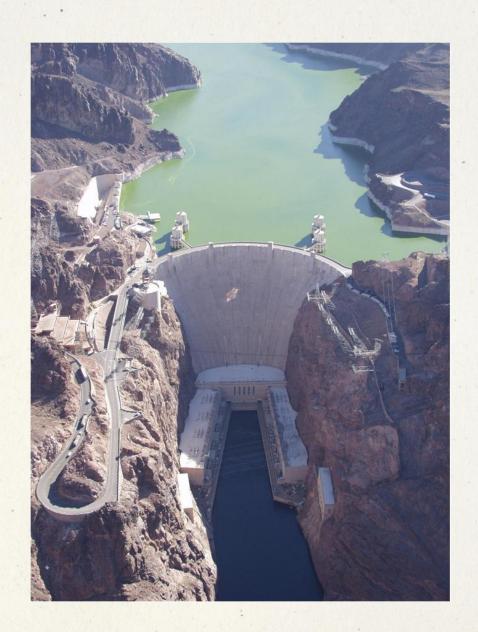
Southern Nevada Water Authority

Project Manager – Applied Water Quality Research

Lake Mead Ecosystem Monitoring Workgroup November 13, 2014 – 1:00 PM

### **Presentation Outline**

- Background on Cyanobacteria
- Cell Damage and Lysis
- MIB, Geosmin, Microcystin Release
- Summary
- Questions



### Frequency of Cyanobacteria Blooms

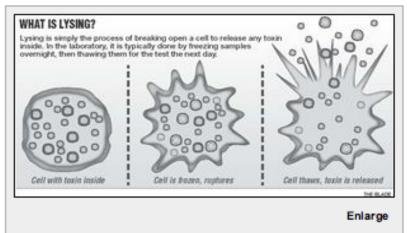
- Increased nutrient loading (i.e. wastewater, agricultural runoff)
- Climate change (i.e. temperature, hydrology)
- Quagga mussel infestation (e.g. selective filtering)





Think of lysing as the act of cracking open algal cells to get any toxin inside released.

In the case of microcystis, Lake Erie's most prevalent form of cyanobacteria, the goal is to get out any toxin that might be inside of it, called microcystin.



Cyanobacteria, also known as harmful blue-green algae, aren't actually algae.

But most people think it is because the bacteria look like and act like algae.

In the pretreatment process, a chemical called potassium permanganate is added at Toledo's waterintake crib so the raw lake water is easier to treat when it arrives at the city's Collins Park Water Treatment

Plant about 6 to 12 hours later.

In the lab, samples of the finished product — the tap water the city is about to distribute — are typically frozen overnight, then thawed the next day to be tested.

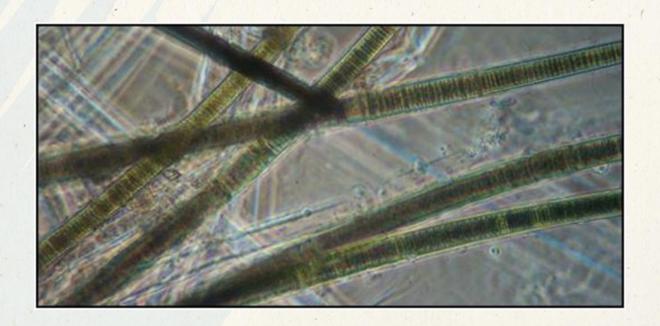
That lyses, or cracks open, any cells that might have eluded the treatment process and still have microcystin inside of them.

The lysing process was developed about 30 to 35 years ago, when cyanobacteria research was in its infancy, said Dave Deardorff, vice president of marketing and sales for Abraxis LLC, a company in Warminster, Pa., that manufactures the microcystin-detection kits many labs use.

### **Background on Cyanobacteria Treatment Research**

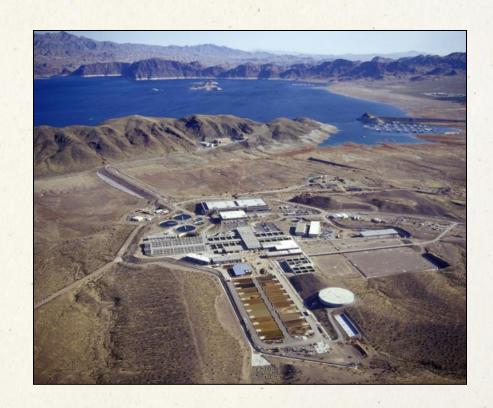
Treatment of <u>extracellular</u> metabolites has been studied for decades

 Minimal research on the release of <u>intracellular</u> (or cell bound) metabolites during oxidation process in drinking water treatment



### **Preoxidation is Common in Drinking Water Treatment**

- Disinfection
- Control of Invasive Species (i.e. zebra/quagga mussel)
- Biofilm Control on Intake Pipelines
- Inorganic Contaminants
  - Iron/Manganese
- Organic Contaminants
  - Taste and Odor Compounds
  - Micropollutants



### Water Research Foundation – Project #4406

- Tailored Collaboration Project
  - Southern Nevada Water Authority
  - University of Colorado at Boulder
  - Fluid Imaging Technologies



- 3 Primary Focus Areas
  - Cyanobacteria Cell Integrity
  - Release of Metabolites (i.e. cyanotoxins, taste/odor compounds)
  - Release of Disinfection Byproduct Precursors

### Water Research Foundation – Project #4406



Tailored Collaboration

Release of Intracellular Metabolites from Cyanobacteria During Oxidation Processes Research conducted from 2011-2013

 Final Report Issued in May 2014 by the Water Research Foundation

Web Report #4406



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### **Chapter 1: Introduction**

Microcystin-LR Oxidation Kinetics (Source: Rodriguez, et. al., 2007)

• Ozone:  $k_{app} = 4.1 \times 10^5 \text{ M}^{-1} \text{s}^{-1}$  Fast

Hydroxyl Radicals: k<sub>app</sub>=1.1 x 10<sup>10</sup> M<sup>-1</sup>s<sup>-1</sup> Fast

■ Chlorine: k<sub>app</sub>=33 M<sup>-1</sup>s<sup>-1</sup> Intermediate

■ Chlorine Dioxide: k<sub>app</sub>=1 M<sup>-1</sup>s<sup>-1</sup> Slow

■ Chloramine: k<sub>app</sub>< 1 M<sup>-1</sup>s<sup>-1</sup> Very Slow

MIB/Geosmin Oxidation Kinetics (Source: Peter and von Gunten, 2007)

• MIB:  $k_{O3}=0.35 \text{ M}^{-1}\text{s}^{-1}, k_{HO}=5.1 \text{ x } 10^9 \text{ M}^{-1}\text{s}^{-1}$ 

■ Geosmin:  $k_{O3}=0.10 \text{ M}^{-1}\text{s}^{-1}$ ,  $k_{HO}=7.8 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ 

FCl<sub>2</sub>/ClO<sub>2</sub>/NH<sub>2</sub>Cl regarded as ineffective

(Source: Glaze, et.al. (1990), Lalezary et.al. (1986))

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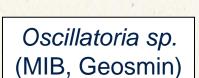
### **Chapter 2: Materials and Methods**

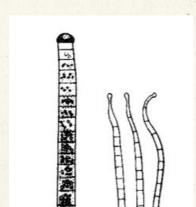
- Selection of Cyanobacteria
  - 1. Availability of an axenic culture
  - 2. Occurrence in source water supplies
  - 3. Ability to produce metabolites of interest
  - 4. Cell morphology

*Microcystis* 

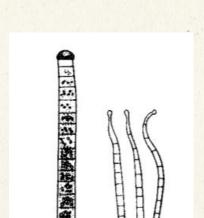
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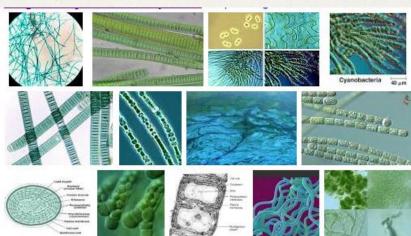
(microcystin-LR)

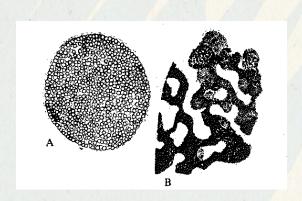


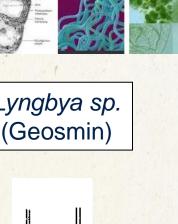










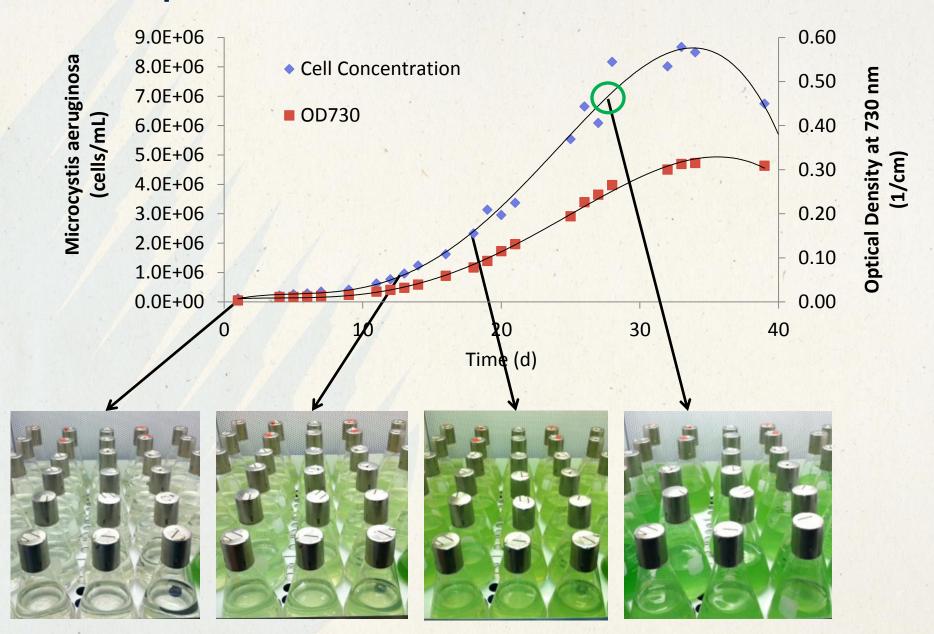


### **Culturing of Cyanobacteria**

- Algal growth chamber
  - 12 hrs light/12 hrs dark
  - Constant temp = 22° C
  - Light Intensity = 2400 lux
- Culturing Methodology
  - 500 mL Erlenmyer Flasks
  - Growth Media (BG-11 or BOLD 3N)
- Growth Curve Development
  - FlowCAM
  - Optical Density at 730 nm



### **Development of Growth Curve**

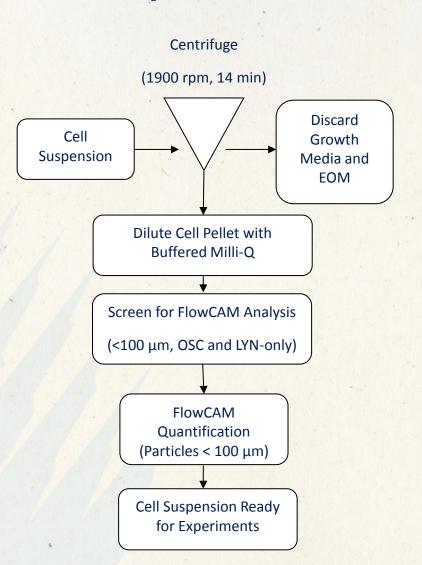


### **Preparation of Cell Suspension**











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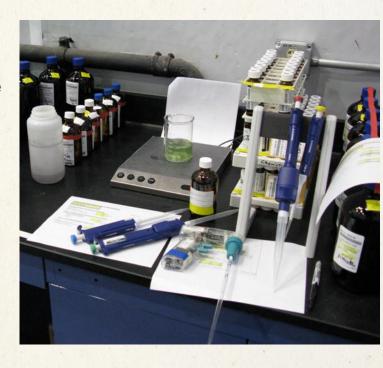
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Conclusions..

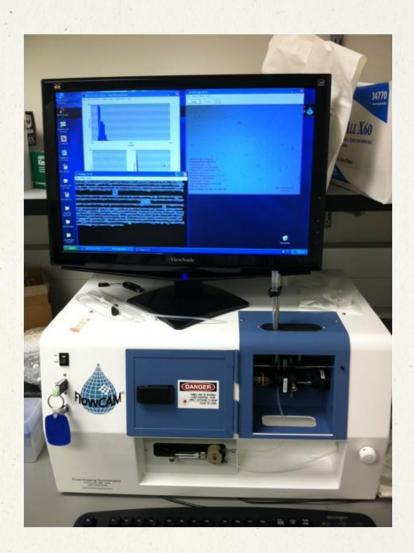
### **Chapter 3: Cell Integrity**

- Added Cell Suspension to Colorado River Water (CRW)
  - Microcystis aeruginosa (MA) 50,000 and 200,000 cells/mL
  - Oscillatoria sp. (OSC) 1,600 and 3,200 cells/mL
  - Lyngbya sp. (LYN) 800 and 1,600 cells/mL
- Oxidation Experiments
  - Ozone, Chlorine, Chlorine Dioxide, Chloramine
  - 5 Dosages of Each Oxidant
  - Residual Measurements

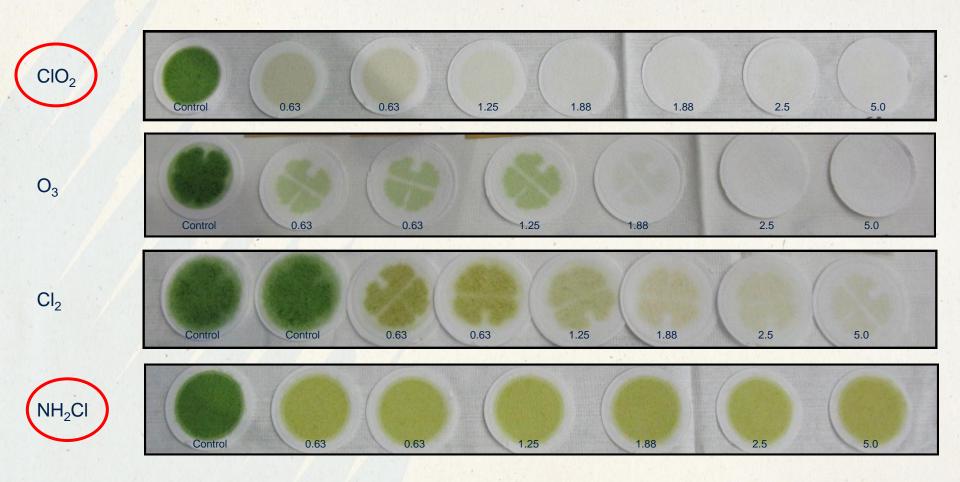


### Cell Damage and Lysis - Approach

- Quantitative Assessment
  - Cell Damage
    - FlowCAM: Trigger Mode (TM)
    - Chlorophyll-a
  - Cell Lysis or Fragmention
    - FlowCAM: AutoImage Mode (AIM)
- Qualitative Assessment
  - FlowCAM: Digital and Binary Images



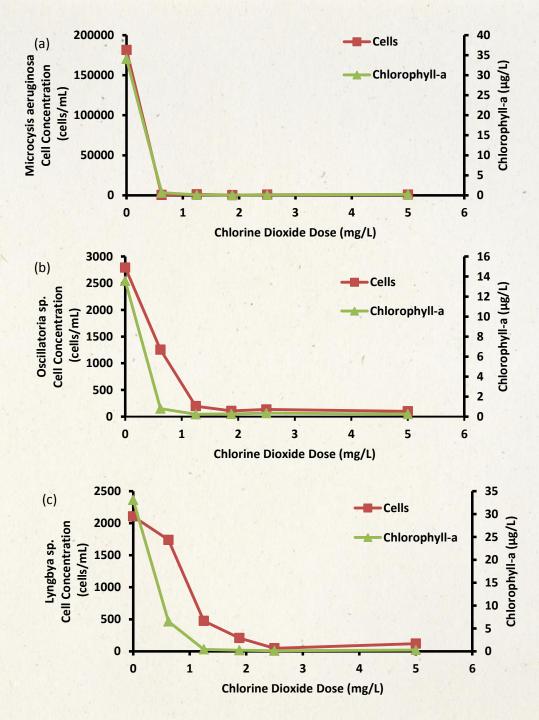
## Degradation of Chlorophyll-a Microcystis aeruginosa (200,000 cells/mL)



### Cell Damage by CIO<sub>2</sub>

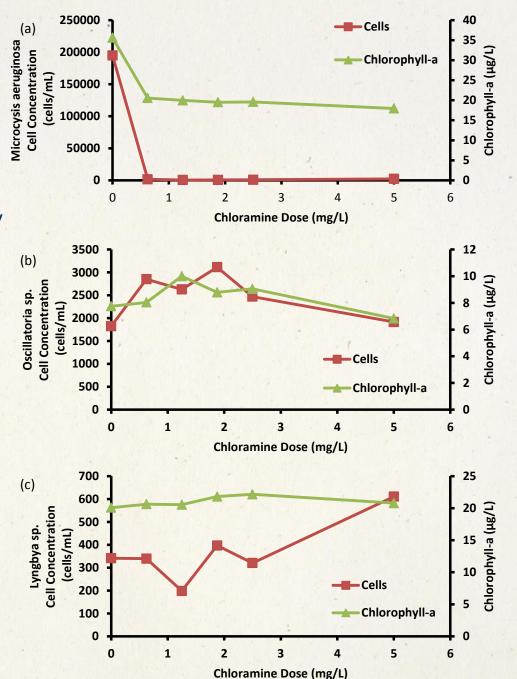
- Assessment
  - Trigger Mode Data
  - Chlorophyll-a

- MA rapidly damaged
  - (CT<386 mg-min/L)</li>
- Dose response
  - OSC and LYN



### Cell Damage by NH<sub>2</sub>Cl

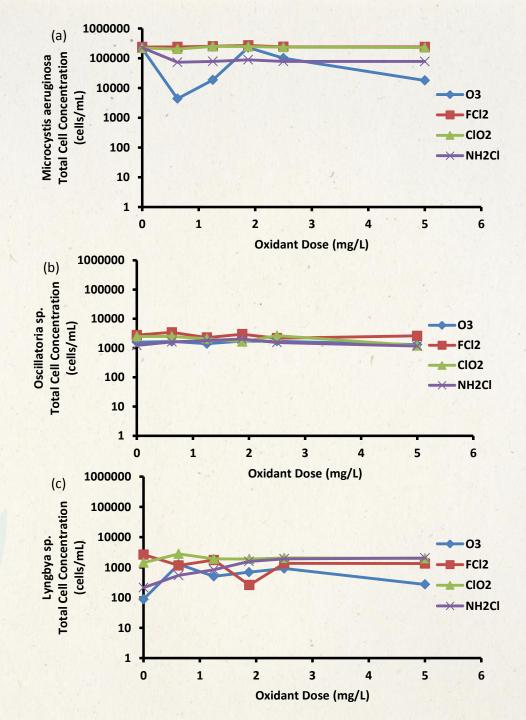
- MA rapidly lost cell integrity
  - CT<612 mg-min/L</li>
  - Diffusion or reaction rate limited
  - Loss fluorescence intensity may have prevented triggering
- OSC and LYN
  - Photosynthetic pigments were unchanged
  - Minimal cell damage by NH<sub>2</sub>Cl



### **Cell Lysis**

- Assessment
  - Auto Image Mode
  - All Particles

 Results indicate cells are damaged without resulting in complete lysis or fragmentation



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### **Metabolite Release - Approach**

Analysis of Filtrate from Oxidation Experiments

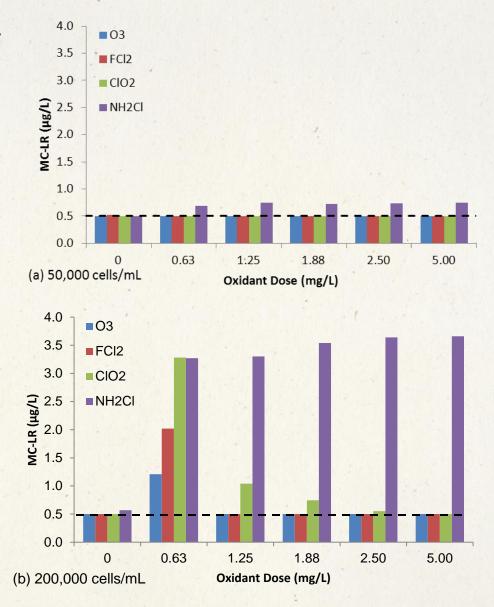
- Testing with Microcystis aeruginosa
  - Release of microcystin-LR
  - World Health Organization (WHO) provisional guideline of 1 μg/L
  - Included on USEPA Candidate Contaminant List 3 (CCL3)
- Testing with Oscillatoria sp.
  - Release of 2-methylisoborneol (MIB)
  - Threshold odor value of 6.3-15 ng/L
- Testing with Lyngbya sp.
  - Release of geosmin
  - Threshold odor value of 1.3-4.0 ng/L

### MC-LR Release from MA

 Minimal MC-LR release occurred with 50,000 cells/mL

- MC-LR with 200,000 cells/mL
  - Release exceeded WHO guideline
  - Majority of release occurred at dosages < 0.63 mg/L</li>

 Oxidation followed published kinetic information



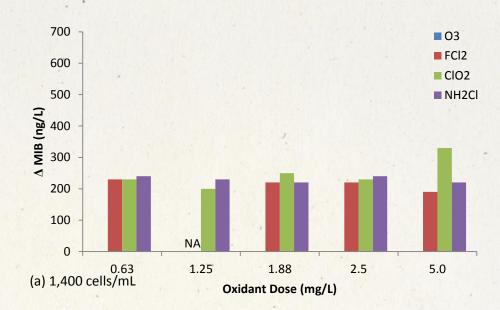
### **MIB Release from OSC**

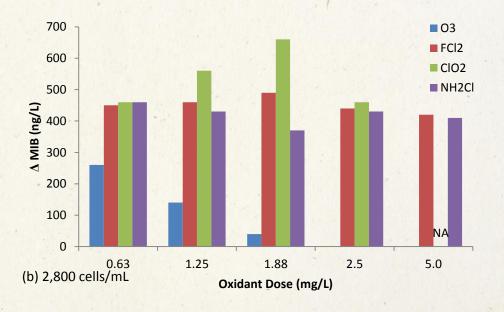
 MIB release exceeded threshold odor values (6.3-15 ng/L)

 Majority of release occurred at dosages < 0.63 mg/L</li>

 Ozone/OH was able to oxidize released MIB

 Release and subsequent oxidation followed published kinetic information



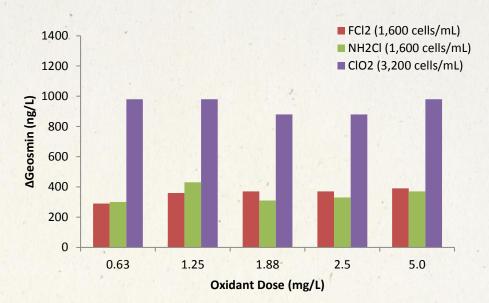


### **Geosmin Release from LYN**

 Geosmin release exceeded threshold odor values

 Ozone/OH was able to oxidize released geosmin

 Release and subsequent oxidation followed published kinetic information



### **Summary of Cell Integrity/Metabolite Release**

Ozone, Chlorine, and Chlorine Dioxide were able to damage all 3 species

Chloramines damaged MA, but not OSC or LYN

Oxidation did not result in complete cell lysis or fragmentation

- All oxidants resulted in the release of metabolites at low exposures
  - Physical release or due to oxidative stress
- Oxidation of extracellular metabolites dependent upon kinetics
  - Ozone and chlorine were able to oxidize released MC-LR
  - Hydroxyl radicals effective for MIB/geosmin

### **Acknowledgements**

- Water Research Foundation
  - Project Manager: Djanette Khiari
  - PAC Members: Jeff Neemann, Aaron Dotson, Karie Holtermann, Lenore Tedesco
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  - Jonathan Dawson, Harry Nelson

